AWARD NUMBER: W81XWH-13-1-0324

TITLE: Improved healing of large, osseous, segmental defects by reverse dynamization: Evaluation in a sheep model.

PRINCIPAL INVESTIGATOR: Christopher H. Evans

CONTRACTING ORGANIZATION: Mayo Clinic
Rochester, MN 55905

REPORT DATE: December 2017

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2017	Final	9-30-2013 – 9-29-2017

4. TITLE AND SUBTITLE
Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0324

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Christopher H. Evans, Ph.D.

E-Mail: evans.christopher@mayo.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Mayo Clinic
200 First Street, SW
Rochester, MN 55905

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project was designed to determine whether reverse dynamization promoted the healing of critical size defects in sheep, as demonstrated in our earlier rat studies. It had two components. In Specific Aim 1,a novel external fixator was designed and subjected to rigorous testing to establish its mechanical properties and suitability for reverse dynamization. This was supplemented by finite element analysis and the use of a strain gauge. This aim was successfully completed, with the external fixator providing 293 N/mm stiffness in the low stiffness position, and 562 N/mm in the high stiffness position. In Specific Aim 2 it was intended to use a 3 cm tibial defect model to compare healing under conditions of high and low stiffness, dynamization and reverse dynamization. However, persistent hardware failures required the sheep to be euthanized. This occurred whether or not the sheep were supported by a sling. Strain gauge data suggested that the peak cycle forces across the defect normally remained below 500N which, according to our mechanical testing data, was well within the capability of the fixator to support. However, CCTV data and strain gauge data suggest that the sheep would sporadically kick, and this led to catastrophic failure of the metal-ware.

15. SUBJECT TERMS
Sheep; tibia; external fixator; dynamization, strain gauge, fracture mechanics

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U Unclassified	U Unclassified	U Unclassified	UU Unclassified	23	USAMRMC
					19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. INTRODUCTION

Large segmental defects in long bones do not heal well and represent a major clinical problem [1]. INFUSE®, comprising recombinant human bone morphogenetic protein-2 (rhBMP-2) delivered on an absorbable collagen sponge, is used by surgeons to assist the healing of large osseous lesions but the clinical results have been disappointing [2]. Moreover, INFUSE® is very expensive.

It is well established that bone healing is influenced by the mechanical environment [3] [4]. Segmental defects may be stabilized mechanically by an external fixator. There has been much interest in the concept of dynamization, whereby the defect is first stabilized rigidly to initiate healing and then subjected to axial motion (dynamization) to promote the subsequent stages of healing and maturation [5]. This axial motion is transmitted as an axial strain or interfragmentary movement (IFM) through the separated bone cortices (fracture gap).

In research funded by a CDMRP Idea Development Award, we used a rat segmental defect model to show that healing in response to rhBMP-2 could be accelerated and improved by “reverse dynamization” in which the fixator is first applied in a loose configuration and then stiffened once bone formation had started [3],[6],[7].

The present research was intended to determine whether reverse dynamization is also effective in sheep, as a stepping stone towards human, clinical trials.

2. KEYWORDS

Bone healing; segmental defect; reverse dynamization; sheep; external fixator

3. ACCOMPLISHMENTS

- **What were the major goals of the project?**

This project had two major goals.
The first was to design and mechanically test a novel external fixator for the sheep tibia that would allow reverse dynamization. This is a new concept in bone healing whereby the defect site is first fixed at low axial stiffness to accelerate the formation of soft callus and then shifted to high axial stiffness to promote endochondral ossification.
The second major goal was to use the external fixator to determine whether reverse dynamization accelerated the healing of a 3 cm, critical size defect in the sheep tibia.

- **What was accomplished under these goals**

<u>Fixator design and mechanical testing</u>
The fixator comprises two 171 mm, aluminum half-bars (Figure 1) screwed together to form an integral external fixator bar with pin-clamping function. There are 39 possible pin positions, with pins entering the bone at either 90 degrees (perpendicular) or 60 degrees (angled). Each bone fragment receives 3 pins, one of which is angled (figure 1). The half-bars clamp only the perpendicular pins, leaving 0.5mm play around the angled pins. This provides fixation of relatively low stiffness. When shims are inserted along the angled pins and clamped, the stiffness of the construct increases. The shims can be inserted and the pins clamped in a painless manner while affixed to the animal. The pins are standard 4 mm titanium pins that are already used clinically to fix long bone fractures.

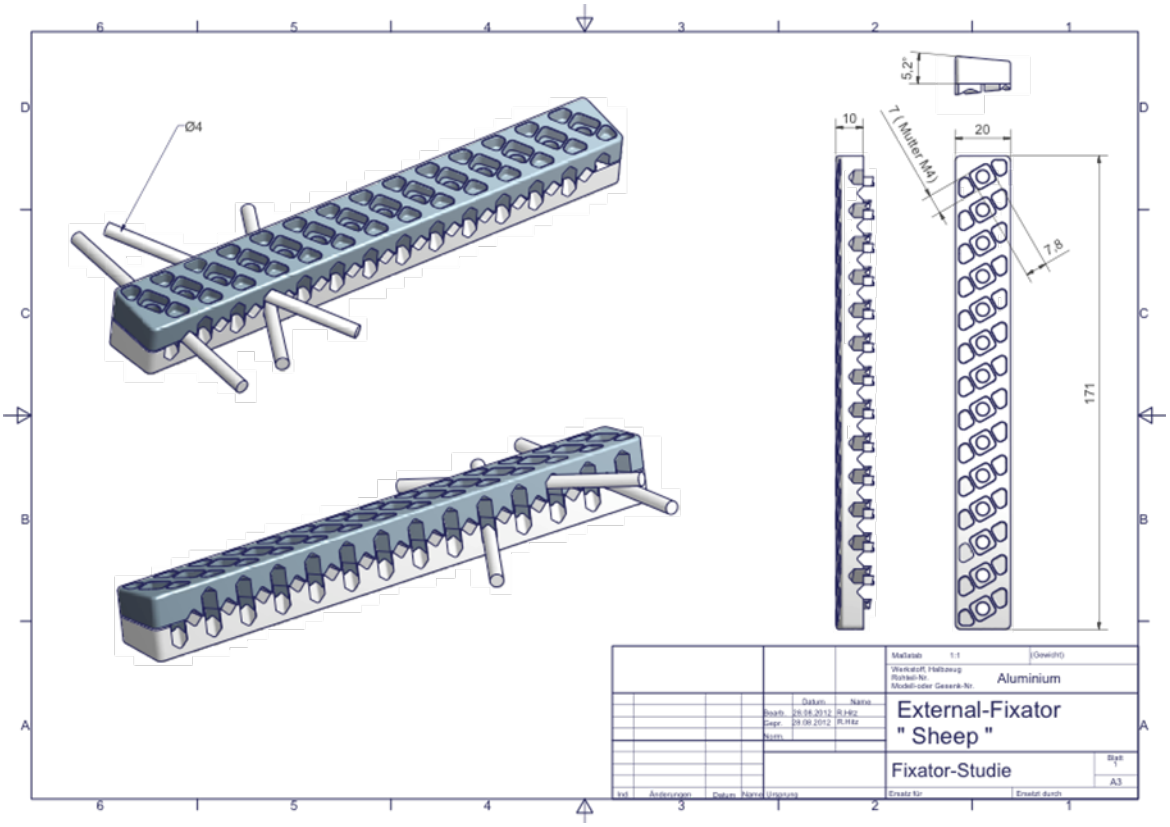


Figure 1. A technical drawing of the adjustable stiffness external fixator for use in a sheep tibial critical-size defect model.

The fixator was tested in a MTS multiaxial testing machine with universal fixture mounts on both “sawbones” and cadaveric sheep tibiae. Testing was taken to a maximum of 500 N axial compression (10 mm/min), which is the greatest possible force generated during the full ovine gait cycle, according to the literature [8]. The data demonstrated that up to 500 N axial loading, the fixator remained within the elastic deformation region; (i.e. the fixator was not permanently deformed). Axial stiffness across the sawbone (SB) or bone samples (B) increased from a stiffness of 293 N/mm when the shims were not inserted (“shims out”), to a stiffness of 562 N/mm when the shims were inserted (“shims in”) (Figure 2).

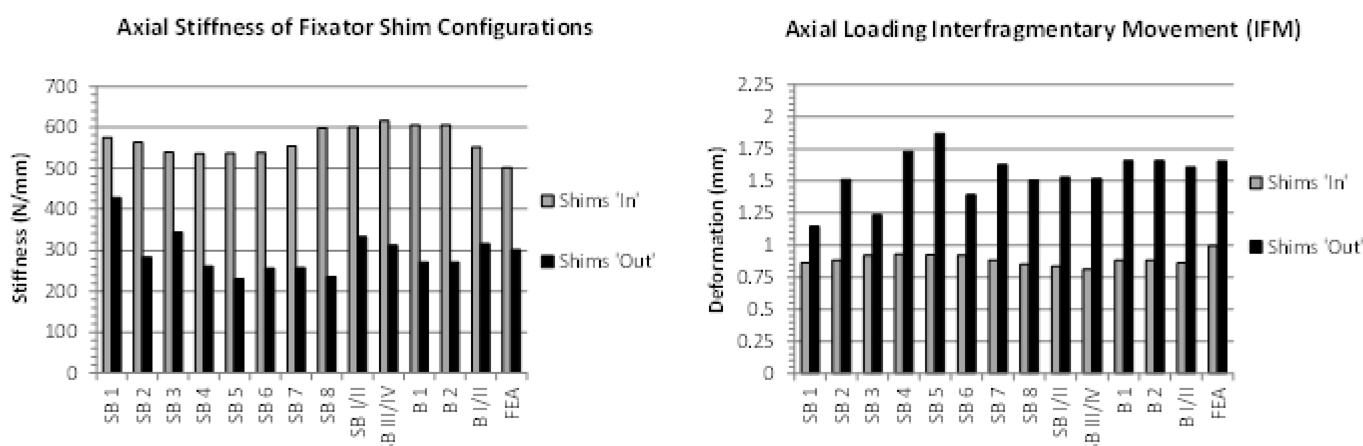


Figure 2. Elastic axial testing of external fixation configurations modelled sawbones and sheep tibiae cadaver samples. Right: Inter-fragmentary movement (IFM) of segmental defect. Left: Fixator stiffness.

Fixator plastic deformation testing was conducted at 10 mm/ min to 1000 N. Ultimate tensile strength and failures were not reached (Figure 3). Elastic yield points were determined for “shims out” (loose) as 510 N and for “shims in” (stiff) as 550 N (Figure 3).

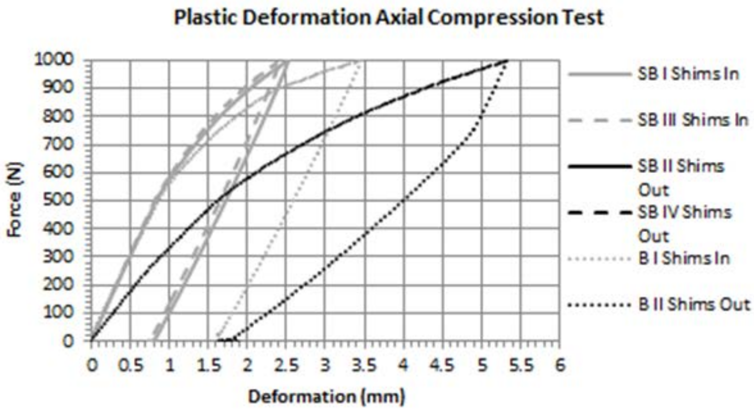


Figure 3. Axial testing of external fixators modeled on a sawbone (SB) and bone (B).

During plastic deformation compression testing, the fixator was fitted with extensometers to assess flexural deformation. The fixator was assumed to deform over the ‘neutral axis’ (the center of the fixator). The elastic deformation region, as determined earlier, showed negligible plastic deformation based on the linear nature of force - deformation plot (Figure 4). Flexural deformation showed slightly increased proximal aspect bending.

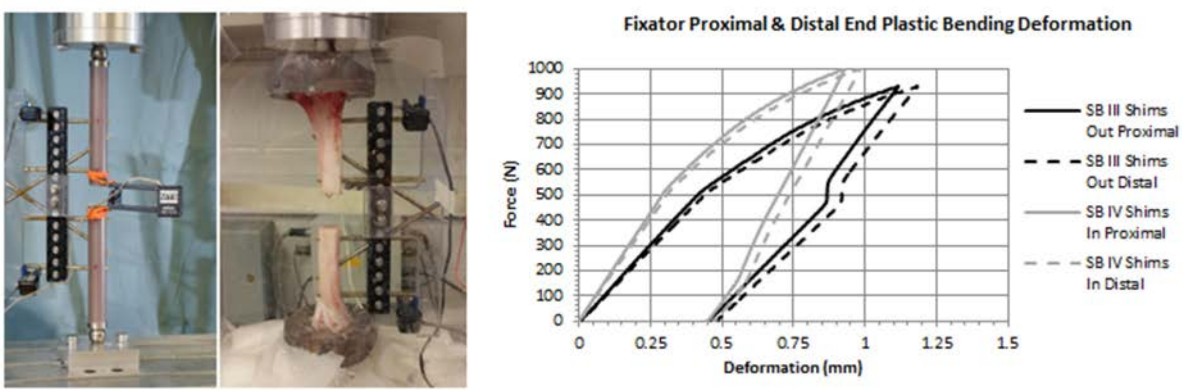


Figure 4. Fixator flexural deformation analysis (left). Sawbone and bone testing samples showing extensometers attached by clear perplex piece bolted to medial plane on fixator. Right: Deformation of proximal and distal aspects of fixator during plastic deformation compression testing.

Axial cyclic testing was conducted for both experimental groups on sawbones and cadaveric sheep tibiae when dynamized (high → low stiffness) and reverse-dynamized (low → high stiffness). The test was conducted to a maximum of 500 N, 4 Hz, 100,000 cycles to simulate approximately 3 months full weight bearing of animal under the worst condition of no bone healing. The data are shown in figure 5. Following an initial stabilization period in all configurations, all deformation plateaued. All initial cycles matched prior quasi-static elastic deformation values and eventually subsided to provide decreased interfragmentary motion (IFM). Data also showed similar trends in the proximal and distal fixator flexural analysis. Flexural bending plateaued to greater value than prior plastic compression testing; however, no yield point was attained (Figure 5).

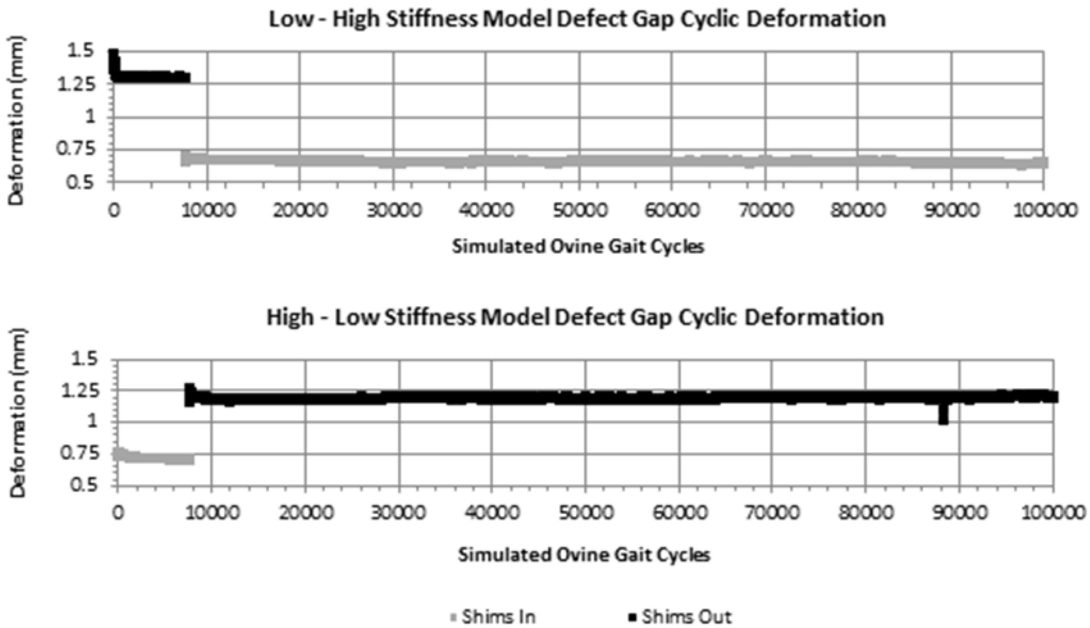


Figure 5. Cyclic axial fatigue testing of external fixators on bone samples across experimental research groups.

The final mechanical testing assessed the torsional stiffness of the fixator configurations. The high stiffness configuration ("shims in") resulted in lower torsional stiffness due to the pin configuration localizing the torsional stress concentration in the fixator (Figure 6).

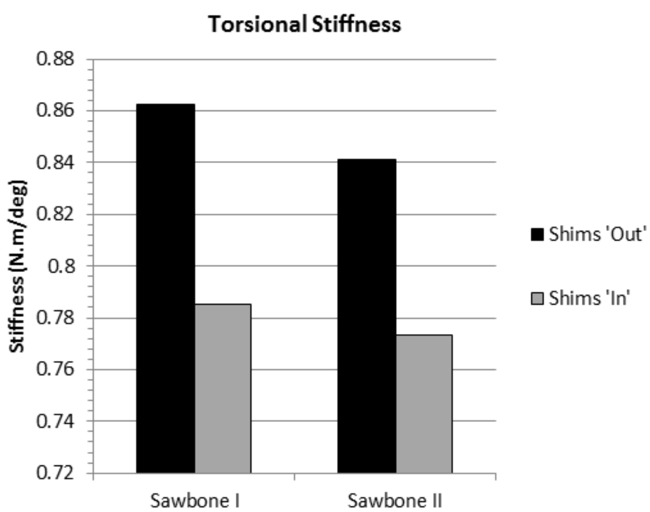


Figure 6. Torsional stiffness testing of sawbone external fixator samples.

Development of a Finite Element (FE) model was conducted through ANSYS 16 to help characterize further the external fixator and enable us to calculate the effects of any future modifications on the assembly frame and its properties. The initial model utilized quadratic tetrahedral elements (390052 elements and 624494 nodes), simplified geometry (no screw threads / fixator 'nuts & bolts') and fixed (bone-screw, fixator bodies) and frictional contact (screw-fixator) conditions. The calculated yielding load and stiffness at low and high-stiffness conditions agreed with mechanical testing results (Figure 7).

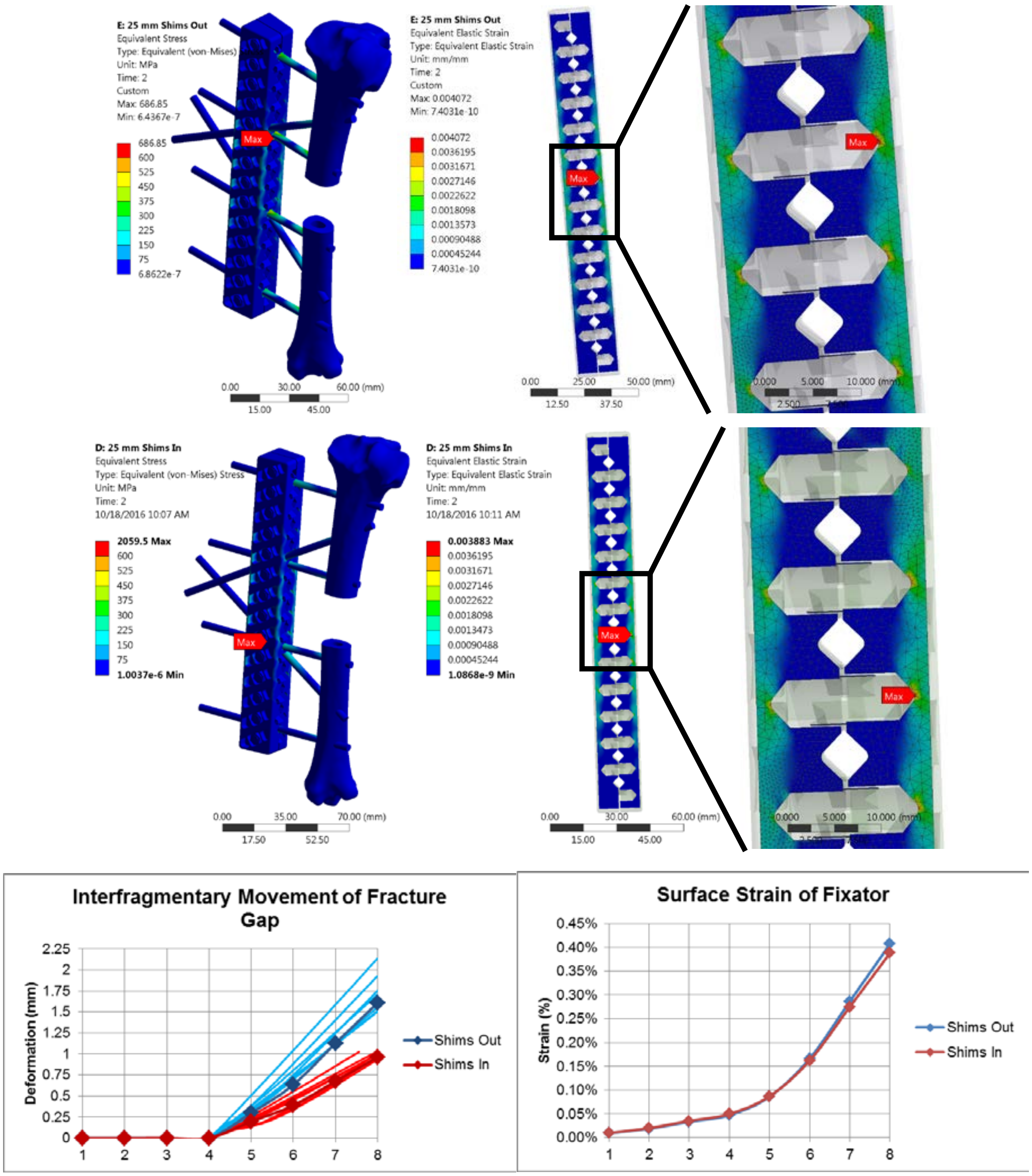


Figure 7. FEA Model of Fixator configurations. Entire stress model and strain distribution of fixator surface for strain gauge characterization. Graph time scale shown in model 'steps' with distal tibial condyle constrained in all directions. IFM graph shows mechanical testing deformation results for model validation.

A collaboration with Michigan Technological University was initiated to explore the implementation of real time monitoring of fracture gap mechanics. This was developed through the addition of a strain gauge system to the fixator surface and wireless signaling technology to monitor voltage outputs through a nearby console. Through mechanical testing and FE modeling, the force through fixator and surface strain could be calculated. This allows for extensive monitoring of the healing process. The system consists of a strain gauge and temperature sensor for calibration of fixator temperature changes (this would affect baseline of strain gauge data), a transmitter unit, a battery unit and an USB receiver unit. The transmitter and battery units are housed in custom printed boxes for protection whilst the gauges covered by a plastic dip (Figure 8)

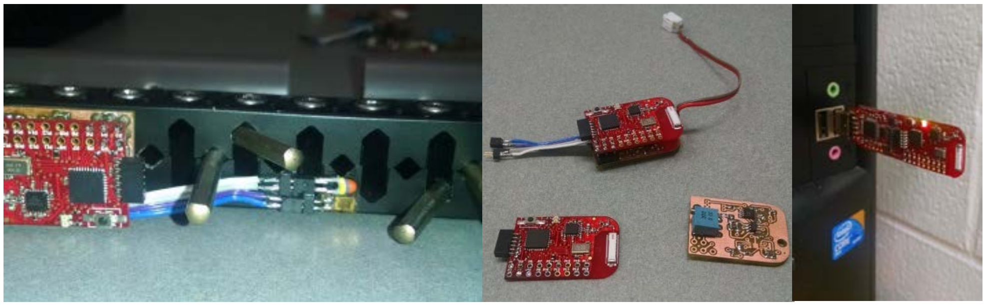
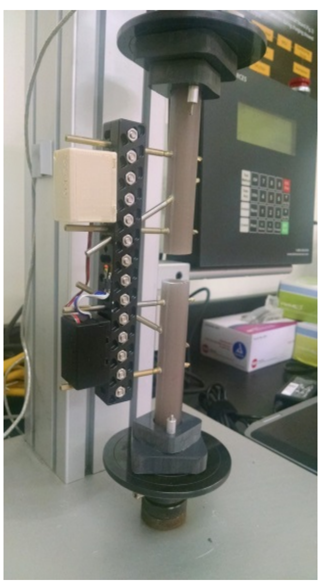


Figure 8 Left: Strain gauge and temperature sensor attached to fixator surface with transmitter unit. Right: Transmitter unit broken down by component layers and USB receiver unit.



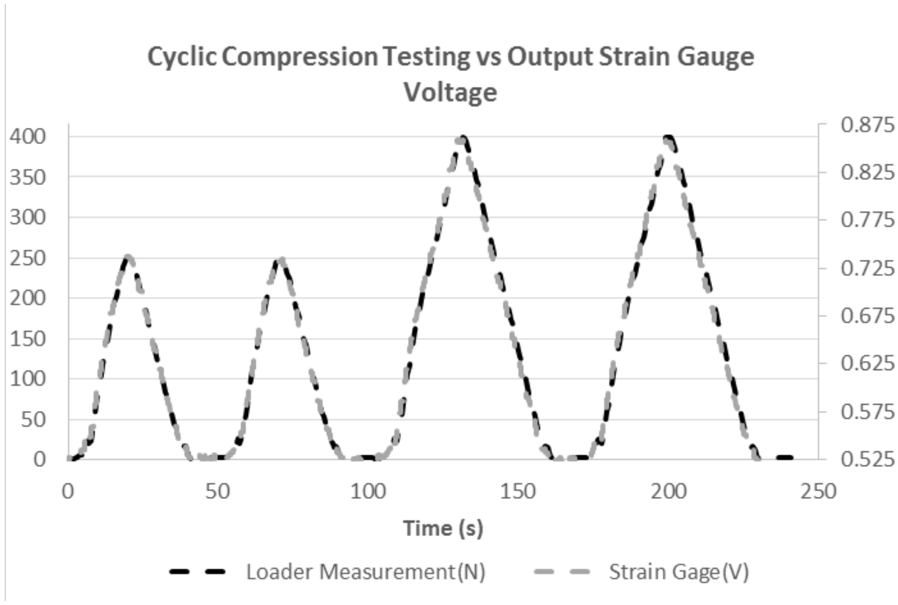


Figure 9, Left: Mechanical testing apparatus for strain gauge characterization. Right: Force applied through mechanical testing with associated strain gauge output voltage during mechanical testing.

Calibration of the gauge was based on mechanical tests assessing the elastic region of the fixator assembly determined through prior testing. A sensitivity range of 0-250N was determined for most accurate measurements and voltage output was measured against force applied during controlled axial tests. Linear slopes for force and associated IFM were determined for voltage conversion. The development of strain conversion was based on mechanical tests assessing the elastic region of the fixator assembly determined through prior mechanical testing. Voltage output

was measured against force applied during controlled axial tests. Linear slopes for force and associated IFM were determined for use in voltage conversion. This was compared against FE model surface strain measurement, corroborated with calculated FE IFM and mechanical testing data to determine output conversions (Figure 9).

Sheep studies

Six sheep were entered into the study. One animal died immediately after surgery from unknown causes, but there were no additional acute deaths after surgery. However, all the operated animals had to be euthanized within eight days because of hardware failure (e.g. Figure 10)

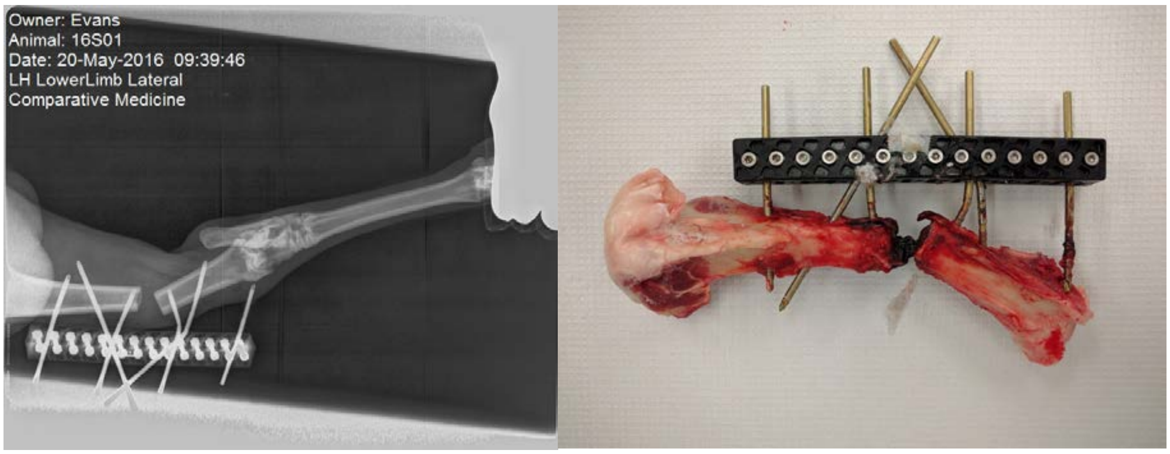


Figure 10; Left: Post-operative day 3 X-Ray after adverse event. Right: Necropsy of tibia with fixator.

These failures were puzzling because the in vivo strain gauge (Figure 11) data did not indicate forces beyond those confirmed as supportable by mechanical testing (i.e., forces were well under 500N; Figure 11; Table 1).

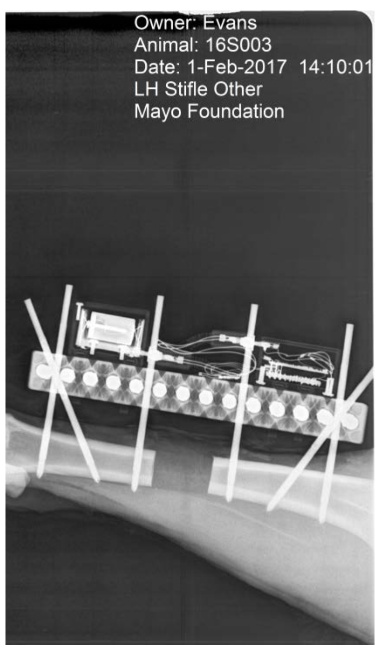


Figure 11. Postoperative x-ray showing successful ovine critical size defect surgery with new fixator configuration / hardware and associated strain gauge circuitry.

Days	Hours Active (Hr)	% Time Active	Baseline Shift (N)	Baseline STD (N)	Peak Step Force (N)	Mean Peak Step Force (N)	Mean Stand Force (N)	# Steps	Steps / Hour
1	8.55	0.70	56.05	10.73	392.03	53.59	3.30	77	9
2	12.98	0.54	130.42	27.69	342.25	66.92	-153.73	326	25.11
3	9.83	0.56	46.52	14.65	335.35	60.10	28.82	425	43.21
4	4.89	0.76	29.28	7.09	257.13	51.09	44.02	273	55.79
5	16.26	0.81	47.38	11.95	395.93	54.91	18.41	879	54.05
6	6.97	0.44	14.46	2.91	109.76	38.52	9.06	39	5.60
7	13.80	0.57	29.76	6.31	107.32	38.90	-6.02	118	8.55
8	3.17	0.37	10.33	2.78			-0.93	0	0

Table 1. Strain gauge data summary showing daily mechanical data.

Nevertheless, we constructed slings to support the animals postoperatively and supplemented strain gauge measurements with 24-hour closed circuit TV surveillance (Figures 12, 13,14).

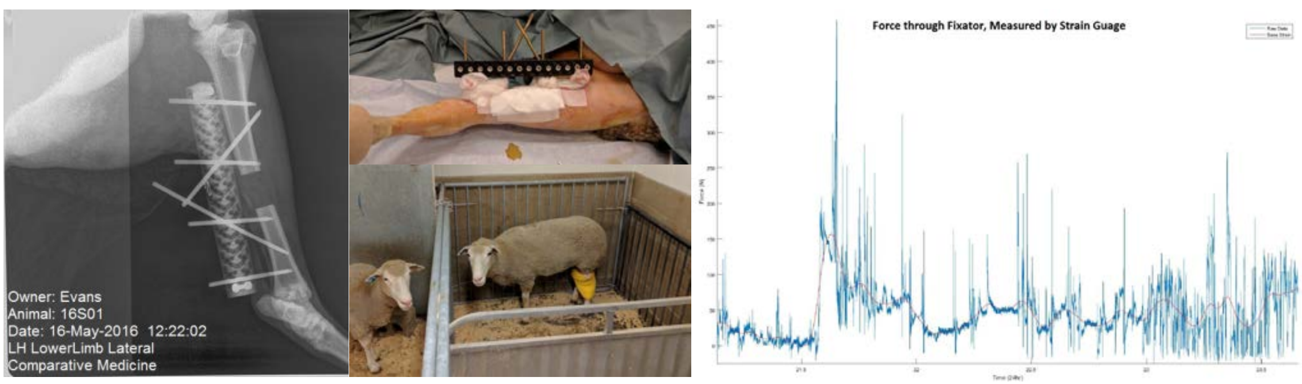
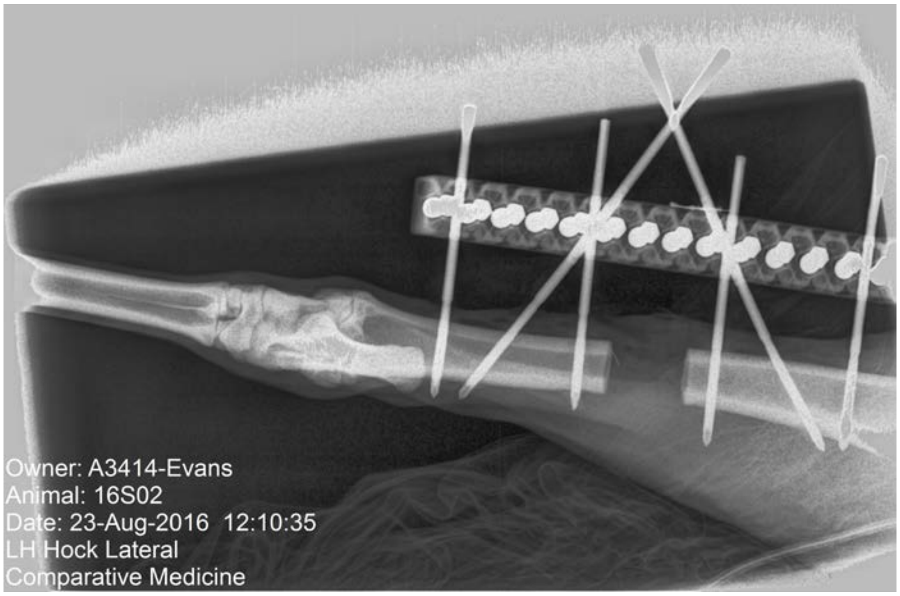
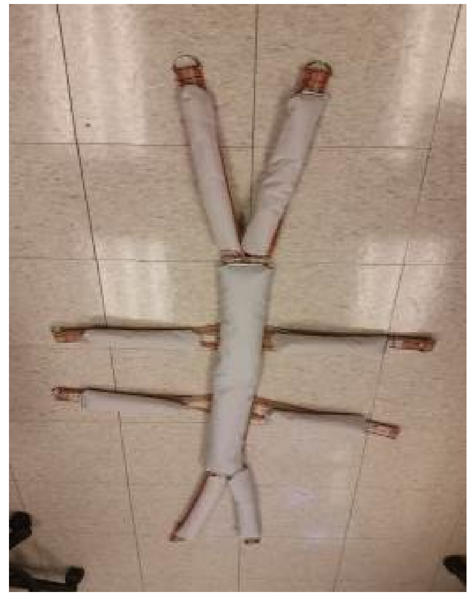


Figure 12, Left: Animal 1 post-operative x-ray. Center top: Post-operative image of external fixator and segmental tibial defect, wound closed. Center bottom: Animal 1 day 2 post-operative, bearing weight and free from major pain identifiers. Right: Fixator strain recorded through wireless strain gauge 6 hours post-surgery.





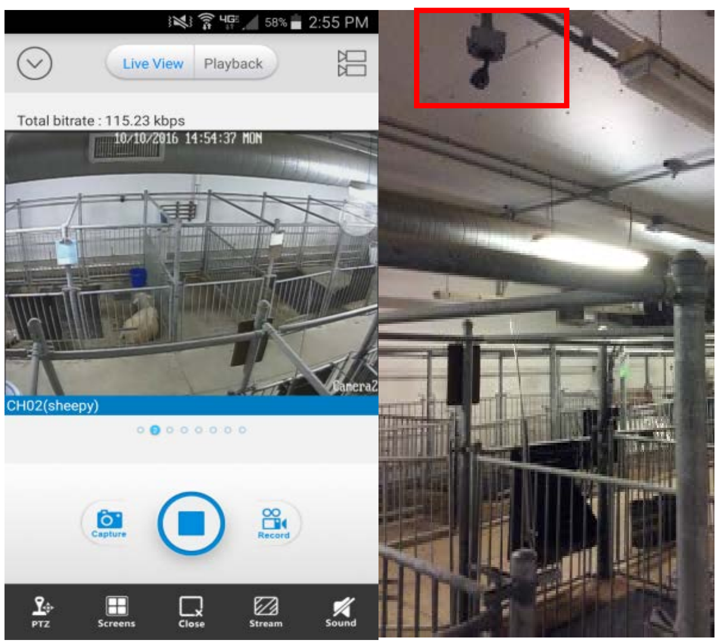


Figure 14; Left: Image capture of CCTV live video monitoring on mobile device. Right: New pens for animal holding and cameras installed for real time monitoring.

Figure 15, Left top and bottom: Post-operative x-rays. Right top and bottom: Day 1 slinging of animal at rest and non-weight bearing.

Figure 16. X-ray showing proximal pin pullout and pin failures.

Days	Hours Active	% Time Active	Baseline Shift (N)	Baseline STD (N)	Peak Step Force (N)	Mean Peak Step Force (N)	Mean Stand Force (N)	# Steps	Steps / Hour
1	3.09	0.92	10.45	2.93	323.65	96.75	109.21	349	9
2	13.44	0.56	44.29	9.25	338.97	88.45	57.59	2163	25.11
3	15.85	0.66	134.07	28.68	490.49	142.35	44.80	3124	43.21
4	13.48	0.56	805.61	321.73	1087.29	142.62	17.85	2491	55.79
5									54.05
6									5.60
7	5.78	0.56	247.32	48.42	389.34	152.00	-68.66	841	8.55
8	5.84	0.54	140.08	34.08	786.95	225.62	21.98	642	0

Table 2 Strain gauge data summary of animal showing daily activity and step forces. High peak forces are highlighted in yellow.

It transpired that failures (e.g. Figure 16) occurred because of high instantaneous forces (>500N) generated when the animals became startled and "kicked" or, in one case, when the sheep was rising from a lying position. Table 2 summarizes readings taken over an 8 day period for one sheep that underwent metal-ware failure after 8 days. Two instances of high peak loading are highlighted in yellow. In response to these results, we re-configured the pin placement on the fixator to provide higher stiffness. These modifications provided a high stiffness of 846 N/mm with 0.59 mm IFM and a low stiffness of 528 N/m with, 0.95 mm IFM. However, metal-ware failures continued to occur and sheep had to be euthanized.

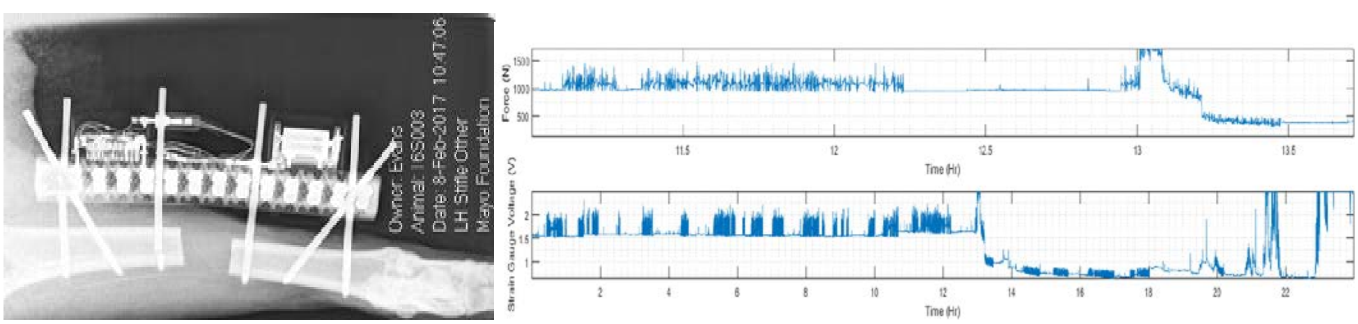


Figure 17: Left: Day 7 x-ray showing secondary perpendicular proximal pin failure. Right: Strain gauge data showing (above) force during pin failure moment [baseline shift +1000N] and (below) associated voltage showing entire day activity

Because of the high mortality rate, the sheep studies had to be discontinued.

- **What opportunities for training and professional development has the project provided?**

The engineer working on this project, Mr. Nicholas Quirk, has registered for a Master's degree, using the data from this study towards his dissertation.

- **How were the results disseminated to communities of interest?**

Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to report

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

The data confirm that it is possible to develop an external fixator whose stiffness can be adjusted while it is fixed to a bone across a large segmental defect. The in vivo experience suggests that the sheep is not a suitable experimental animal for its further development.

- **What was the impact on other disciplines**

Nothing to report

- **What was the impact on technology transfer?**

Nothing to report

- **What was the impact on society beyond science and technology**

Nothing to report

5. CHANGES/PROBLEMS

- **Changes in approach and reasons for change**

The repeated metal-ware failures when the fixators were applied to the sheep prompted several changes in approach. In terms of post-operative care, we designed and made slings for the animals to reduce post-operative forces on the operated leg. We also installed CCTV cameras to allow 24 hour surveillance of the animals. Moreover, the fixators were instrumented to allow us to measure the in vivo forces across the fixator. We also changed the configuration of the pins used to secure the fixator.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

As this is a final report actual problems and how we dealt with them are mentioned above and described in detail in the text.

- **Changes that had a significant impact on expenditures**

The overall budget was unaffected. The problems in sheep husbandry meant that we allocated more resources than planned towards the post-surgical management and monitoring of the sheep.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

See below

- **Significant changes in use or care of human subjects**

Not applicable

- **Significant changes in use or care of vertebrate animals**

As noted elsewhere in this report, we made significant changes to the use and care of the sheep. These changes included the use of a sling to support the sheep during the post-operative period, 24-hour monitoring by CCTV and the use of a strain gauge to measure in vivo loads. The veterinary staff were kept fully appraised of these changes, and it was not necessary to file amendments to IACUC or ACURO.

- **Significant changes in use of biohazards and/or select agents**

Not applicable

6. PRODUCTS

- **Publications, conference papers, and presentation**
  - **Journal publications**

    V Glatt, S Tepic, CH Evans: Reverse dynamization: A novel approach to bone healing. J Am Acad Orthop Surg 24: e60-e61, 2016

  - **Books or other non-periodical, one-time publications**

    Nothing to report

  - **Other publications, conference papers, and presentations**

    Poster presentations:

    NP Quirk, A Thoreson, RE De la Vega, MJ Coenen, M Trujillo, CM Lopez De Padilla, S Tepic, CH Evans. Characterization of a novel dynamizable external fixator for ovine tibial segmental defects. European Cells & Materials Volume 3, Supplement 3, 56, 2016. http://www.ecmjournal.org/journal/supplements/vol032supp03/pdf/Vol032Supp03056.pdf

    N. P. Quirk, A. Thoreson, R. E. De la Vega, M.D., M. J. Coenen, M.Trujillo, Ph.D, M, Morsey, M.B., B.Ch., A. T. Mohan, MBBS., Y. J. Sur, M.D., Ph.D., S. Tepic, D.Sc., C. H. Evans, Ph.D. Mechanical characterization of a novel external fixator for dynamizing ovine osseous defects. Poster No. 2185. Orthopedic Research Society Annual Meeting, Orlando, FL, USA, March 5-8, 2016

    Oral Presentations:

    Regenerative rehabilitation applied to bone. Invited talk presented at the 4th Annual Symposium on Regenerative Rehabilitation. Atlanta, GA 2016 (Speaker – Evans)

    Low- and high-tech approaches to imp[roving bone healing. Invited talk presented at the 52nd Western India Regional Orthopaedic Conference, Mumbai, India 2017 (Speaker- Evans)

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals worked on the project?**

Name: Christopher H. Evans, PhD
Project Role: Principal Investigator
Nearest person month worked: 3
Contribution to project: Planning, oversight, decision making, administration, project management

Name: Michael Coenen, BS
Project Role: Technician
Nearest project month worked: 2
Contribution to project: Preparation of surgical tools, assisting in surgery, assisting with biomechanical testing

Name: Nicholas Quirk, BS
Project Role: Engineer
Nearest project month worked: 12
Contribution to project: Mechanical testing, remote monitoring, data analysis

Name: Andrew Thoresen, BS
Project role: Engineer
Nearest project month worked: 1
Contribution to project: mechanical testing

Name: Miguel Trujillo, PhD
Project role: Research Scientist
Nearest person month worked: 2
Contribution to project: Data analysis, project management

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

- **What other organizations were involved as partners?**

Organization name: Michigan Technical University
Location of Organization: Houghton, Michigan
Partner's contribution to the project: Engineers at Michigan State University developed the strain gauges that were attached to our external fixators.

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS**

Not applicable. (Soon after the grant was awarded, the PI moved to Mayo Clinic and the partnering PI subsequently moved to Cedar Sinai Medical Center. The functions of the partnering PI were transferred to Mayo Clinic.

9. APPENDICES

Included on following pages

Reverse Dynamization: A Novel Approach to Bone Healing

Vaida Glatt, PhD

Slobodan Tepic, DrSci

Christopher Evans, PhD

From the Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia (Dr. Glatt), Kyon AG, Zurich, Switzerland (Dr. Tepic), and the Rehabilitation Medicine Research Center, Mayo Clinic, Rochester, MN (Dr. Evans).

The authors' work in this area has been supported by the US Department of Defense (W81XWH-10-1-0888 and W81XWH-13-0324), the AO Foundation, Switzerland (S-08-42G), and the Vice-Chancellor's Research Fellowship of Queensland University of Technology, Australia.

Dr. Tepic or an immediate family member has received royalties from Ruetschi Technology; is an employee of Kyon and Scyon Orthopaedics; and has stock or stock options held in Akeso, Kyon, and Scyon Orthopaedics. Dr. Evans or an immediate family member serves as a paid consultant to or is an employee of Orthogen AG and TissueGene; has stock or stock options held in Aldabra, Orthogen AG, and TissueGene; has received nonincome support (such as equipment or services), commercially derived honoraria, or other non-research–related funding (such as paid travel) from Medtronic Sofamor Danek; and serves as a board member, owner, officer, or committee member of the Advanced Equine Research Institute. Neither Dr. Glatt nor any immediate family member has received anything of value from or has stock or stock options held in a commercial company or institution related directly or indirectly to the subject of this article.

J Am Acad Orthop Surg 2016;24: e60-e61

http://dx.doi.org/10.5435/JAAOS-D-16-00239

Julius Wolff (1836-1902) demonstrated the remarkable ability of the long bones to adapt to their mechanical environment. This property underlies the strategy of dynamization as a way to improve bone healing. Introduced by De Bastiani et al[1] in the 1980s, dynamization requires initial rigid stabilization of an osseous defect to allow the soft tissues to recover and bone healing to begin. With the first radiographic indication of callus, which usually occurs after approximately 3 weeks, stabilization is loosened in the axial plane so that load is progressively transferred to the regenerate to stimulate bone formation and maturation.

This article describes a novel strategy in which the defect is first stabilized at low axial stiffness, with subsequent increase in stiffness at the first signs of radio-opacity. We call this reverse dynamization.[2]

Reverse Dynamization Concept

The concept of reverse dynamization arose as a means of stimulating endochondral bone formation. We predicted that early exposure of the defect to loading would enhance the differentiation of mesenchymal progenitor cells into chondrocytes, a process accelerated by mechanical stimulation.[3] Such loading, however, threatens to impair endochondral ossification by disrupting the formation of blood vessels within the ossifying structure. For this reason, we proposed to increase the rigidity of fixation at the first radiologic signs of mineral deposition within the defect. Epari et al[4] subsequently published a theoretical paper supporting our postulates.

Experimental Evidence

First experiments used a rat femoral critical-size diaphyseal defect stabilized with an external fixator. The fixator was designed to allow the axial stiffness to be modulated while attached to a living animal.[5] Recombinant human bone morphogenetic protein-2 (BMP-2) was used to initiate healing. These studies confirmed that healing was accelerated and improved by reverse dynamization using initial low stiffness (114 N/mm) fixation, followed by reverse dynamization to a high stiffness fixator (254 N/mm) after 2 weeks of healing[2] (Figure 1).

In a subsequent publication,[6] we confirmed this phenomenon and began to define the stiffness parameters and BMP-2 dose requirements.

Next Steps

We are about to start exploring the effectiveness of reverse dynamization in a sheep tibial defect model[7] as a prelude to possible human clinical trials and veterinary applications. Meanwhile, the mechanism of action of reverse dynamization requires elucidation. As noted, it was originally proposed as a means of stimulating the endochondral process. However, in our first study,[2] we could detect no evidence of early chondrogenesis. Nevertheless, the subsequent study,[6] using a lower dose of BMP-2 and a wider range of stiffnesses, identified cartilage at the defect site. The mechanism may thus be subtle, possibly involving an effect on the production of inflammatory

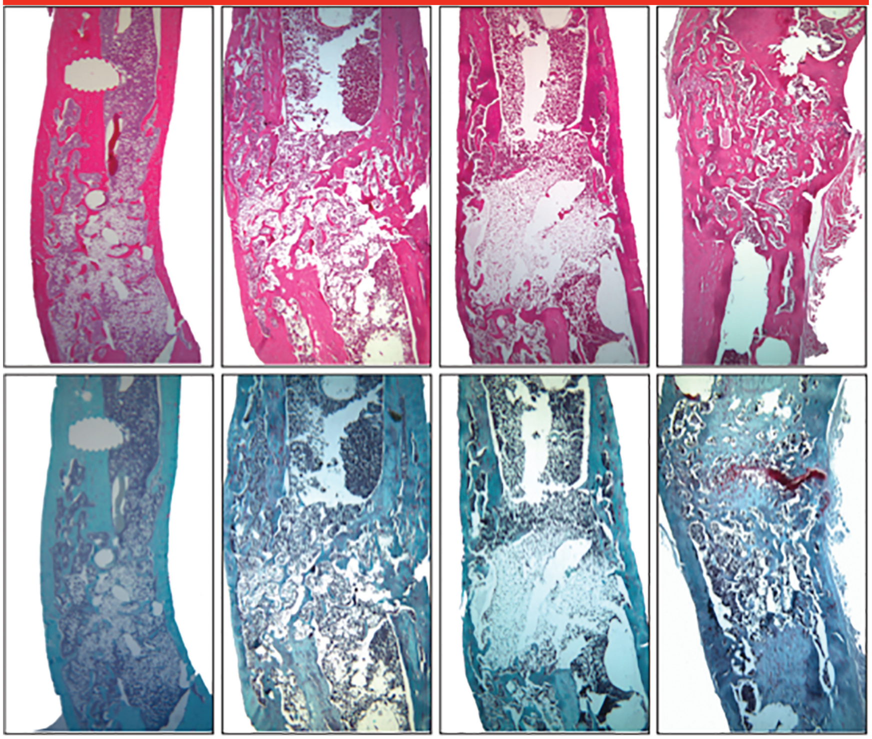
mediators and the activation of the transcription factor nuclear factor kappa-B (NF-κB).[8]

Experiments so far have used a large segmental defect model. The question is whether reverse dynamization will be effective in subcritical size defects and fractures. Pioneering studies by Hente et al[9] suggest effectiveness in the former. These investigators noted a dramatic increase in bone formation at the site of a 2-mm diaphyseal osteotomy in sheep under cyclic compression, but not distraction.

There are no preclinical data concerning the effectiveness of reverse dynamization in fracture healing, but Howard et al[10] recently published a pilot study in which a type of reverse dynamization was used to treat tibial fractures in humans. The outcomes were superior to those normally achieved using standard dynamization.

So far, the empirical evidence concerning reverse dynamization has come from studies using external fixators. Although it is possible to envision the use of sophisticated internal fixation devices for this purpose, external fixation has advantages of simplicity, affordability, and the possibility of removing the fixator once weight bearing is indicated, thus promoting maturation of the regenerate while preventing subsequent stress shielding.

Figure 1



Histologic appearance of defects 8 weeks after stabilization with low-stiffness (ExFixLow), medium-stiffness (ExFixMed), or high-stiffness (ExFixHigh) fixators or subjected to reverse dynamization RD). Low stiffness = 114 N/mm; medium stiffness = 185 N/mm; high stiffness = 256 N/mm. Stiffness increased from low to high after 2 weeks. Top row: hematoxylin-and-eosin staining; bottom row: safranin orange–fast green staining. (Reproduced with permission from Glatt V, Miller M, Ivkovic A, et al: Improved healing of large segmental defects in the rat femur by reverse dynamization in the presence of bone morphogenetic protein-2. *J Bone Joint Surg Am* 2012;94[22]):2063-2073.)

References

References printed in **bold type** are those published within the past 5 years.

1. De Bastiani G, Aldegheri R, Renzi Brivio L: The treatment of fractures with a dynamic axial fixator. *J Bone Joint Surg Br* 1984;66 (4):538-545.
2. **Glatt V, Miller M, Ivkovic A, et al: Improved healing of large segmental defects in the rat femur by reverse dynamization in the presence of bone morphogenetic protein-2. *J Bone Joint Surg Am* 2012;94 (22):2063-2073.**
3. Schätti O, Grad S, Goldhahn J, et al: A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. *Eur Cell Mater* 2011;22: 214-225.
4. **Epari DR, Wehner T, Ignatius A, Schuetz MA, Claes LE: A case for optimising fracture healing through inverse dynamization. *Med Hypotheses* 2013;81 (2):225-227.**
5. **Glatt V, Evans CH, Matthys R: Design, characterisation and in vivo testing of a new, adjustable stiffness, external fixator for the rat femur. *Eur Cell Mater* 2012;23: 289-298.**
6. **Glatt V, Bartnikowski N, Quirk N, Schuetz M, Evans C: Reverse dynamization: Influence of fixator stiffness on the mode and efficiency of large-bone-defect healing at different doses of rhBMP-2. *J Bone Joint Surg Am* 2016;98(8):677-687.**
7. **Quirk N, Thoreson A, De la Vega RE, et al: Poster presentation. Mechanical characterization of a novel external fixator for dynamizing ovine osseous defects. Poster No. 2185. Presented at the Annual Meeting of Orthopaedic Research Society, Orlando, Florida, March 5-8 2016.**
8. Agarwal S, Deschner J, Long P, et al: Role of NF-kappaB transcription factors in antiinflammatory and proinflammatory actions of mechanical signals. *Arthritis Rheum* 2004;50(11): 3541-3548.
9. Hente R, Füchtmeier B, Schlegel U, Ernstberger A, Perren SM: The influence of cyclic compression and distraction on the healing of experimental tibial fractures. *J Orthop Res* 2004;22(4): 709-715.
10. **Howard CB, Leibergal M, Elishoov O, et al: Can changing the mechanical environment increase the speed of fracture healing? A pilot study in tibial fractures. *J Trauma Treat* 2013;2:176.**

Characterization of a novel dynamizable external fixator for ovine tibial segmental defects

Nicholas P. Quirk[1], Andrew Thoreson[1], Rodolfo E. De la Vega, M.D.[1], Michael J. Coenen[1], Miguel Trujillo, Ph.D[1], Consuelo M. Lopez De Padilla, M.D.[1], Slobodon Tepic, D.Sc.[2], Christopher H. Evans, Ph.D.[1]

[1] *Mayo Clinic, Rochester, MN,* [2] *Kyon AG, Zurich, Switzerland*

INTRODUCTION: Large segmental defects in long bones present a clinical challenge to surgeons. There is much interest in the influence of the mechanical environment on the healing of these defects. Dynamization of the fracture gap has been shown to promote the subsequent stages of healing and maturation. This occurs through stiffness modulation of the fixation construct, stabilizing fracture during healing and has been successfully evaluated in rodent models. Prior to large animal study translation, a suitable, adjustable, well-characterized, external fixator is required.

The ultimate goal of using this fixator is to modify the defect mechanical environment in conjunction with recombinant human BMP-2 to improve healing in an ovine tibial segmental defect model.

METHODS: Fixators were characterized through mechanical testing by sawbone and ovine cadaver tibiae samples, and data was used to validate a finite element (FE) model. A 30mm fracture defect and 20 mm 'bone-to-fixator' offset was used on all samples. Extensometers were attached across the defect for inter-fragmentary movement (IFM) and at fixator ends to characterize flexural deformation of the fixator. Plastic and elastic axial compressive testing, torsional testing and cyclic axial testing were performed on the constructs. A FE model was developed using ANSYS and utilized quadratic tetrahedral elements (390052 elements and 624494 nodes), simplified geometry (no screw threads / fixator 'nuts & bolts') and fixed (bone-screw, fixator bodies) and frictional contact (screw-fixator) conditions.

RESULTS: Plastic axial testing showed yielding for low stiffness configuration at 520 N and 550 N for high stiffness. Elastic axial testing showed corroboration between sawbone and cadaveric samples. Elastic axial testing and torsional testing confirmed FE model predictions. IFM exhibited a mean value of 1.526 mm and 0.901 mm for low and high stiffness, respectively, for elastic axial testing. Cyclic fatigue testing showed plateaued deformation across 100,000 cycles for all groups.

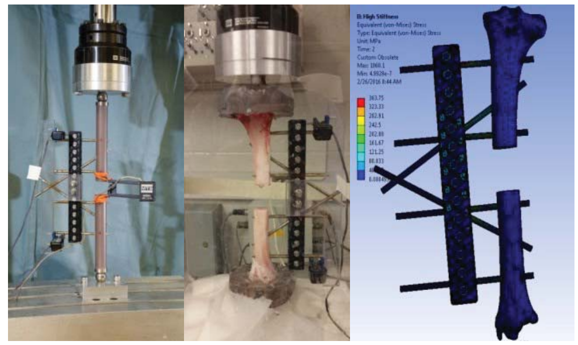


Fig. 1: Methods of characterization for external fixator (left to right); sawbone mechanical testing, cadaveric sample mechanical testing, FE analysis.

DISCUSSION & CONCLUSIONS: Fixator dynamization increased the construct stiffness by approximately 2-fold. Based on prior results from rat models, this is appropriate for enhanced bone healing. Moreover, negligible IFM differences of the fracture gap occurred during repeated load-cycling to mimic the projected lifecycle of the fixator while attached to the sheep. This shows stability of fixator across its life span and efficacy in a weight bearing animal. FE model results were generally in agreement with bench testing in key mechanical properties.

The successful design, manufacture and characterization of this external fixator provides the means to evaluate the efficacy of dynamization in ovine models of bone healing. This fixator may be useful in small animal veterinary practice and could form the basis for a device suitable for use in humans.

ACKNOWLEDGEMENTS: We would like to thank Lawrence Berglund for assistance with mechanical testing. This study was funded as part of a Department of Defense research grant (award number W81XWH-13-1-0324).

Mechanical Characterization of a Novel External Fixator for Dynamizing Ovine Osseous Defects

Nicholas P. Quirk[1], Andrew Thoreson[1], Rodolfo E. De la Vega, M.D.[1], Michael J. Coenen[1], Miguel Trujillo, Ph.D[1], Mohamed Morsy, M.B., B.Ch.[1], Anita T. Mohan, MBBS.[1], Yoo J. Sur, M.D., Ph.D.[1], Slobodon Tepic, D.Sc.[2], Christopher H. Evans, Ph.D.[1]
[1] Mayo Clinic, Rochester, MN, [2]Kyon AG, Zurich, Switzerland

Author Disclosure Information: N.P. Quirk: None. A. Thoreson: None. R.E. De la Vega: None. M.J. Coenen: None. M. Trujillo: None. M. Morsy: None. A.T. Mohan: None. Y.J. Sur: None. S. Tepic: 4; Kyon AG. C.H. Evans: 3B; Orthogen AG; TissueGene Inc.. 4; Orthogen AG; TissueGene Inc..

INTRODUCTION: Large segmental defects in long bones present a clinical challenge for surgeons. There has been much interest in the influence of the mechanical environment on the healing of these defects. Dynamization of the fracture gap has been shown to promote the subsequent stages of healing and maturation. This occurs through modulation of the mechanical stiffness of the fixation construct stabilizing fracture during healing. Dynamization of low-to-high and high-to-low stiffness modulations have been successfully evaluated in rodent models. Prior to large animal study translation, a suitable, adjustable, well-characterized, external fixator is required. This communication describes the mechanical characterization of such a novel external fixator *(Figure 1a)*, conducted through bench mechanical testing and finite element analysis (FEA). This method allows for an in-depth investigation into the fixator's mechanical properties and prediction of response to theoretical loading scenarios. The fixator adjusts for both 'low' and 'high' axial stiffness through 'shim' insertion adjacent to angled pins. Key design considerations were ease of manufacture and surgical implementation. The ultimate goal of using this fixator is to modify the defect mechanical environment in conjunction with recombinant human BMP-2 in order to improve healing in an ovine tibial segmental defect model. Group models for subsequent animal study are: 1) low stiffness, 2) high stiffness, 3) low-to-high stiffness, 4) high-to-low stiffness.

METHODS: Fixators were characterized through mechanical testing using sawbone and ovine cadaver tibiae samples, and data was used to validate a finite element (FE) model. A 30mm fracture defect and 20 mm 'bone-to-fixator' offset was used on all prepared samples. Extensometers were attached across the defect to assess inter-fragmentary movement (IFM) and at fixator proximal/distal ends to characterize flexural deformation of the fixator. Plastic (10mm/min; n = 4; 2 sawbone, 2 cadaver) and elastic axial compressive testing (0.25 mm/sec; n = 8; 6 sawbone, 2 cadaver), torsional testing (0.33 deg/sec; n = 2 sawbone) and cyclic axial testing (4Hz, 50N – 500N, 100,000 cycles; n = 4; 2 sawbone, 2 cadaver) were performed on fixator-bone constructs. Construct hysteresis was accounted for through 3 repeated tests per sample for elastic axial and torsional testing. Construct stiffness for high and low-stiffness conditions were evaluated and maximum construct deflection was assessed. A FE model was developed using ANSYS and utilized quadratic tetrahedral elements (390052 elements and 624494 nodes), simplified geometry (no screw threads / fixator 'nuts & bolts') and fixed (bone-screw, fixator bodies) and frictional contact (screw-fixator) conditions. Yielding load and stiffness at low and high-stiffness conditions were compared to FE results.

RESULTS: Plastic axial testing showed yielding for low stiffness configuration at 520 N and 550 N for high stiffness configuration. To confirm that the fixator could bear the forces imposed by adult, ambulating sheep (peak gait force ~ 450 N), a 500 N load was chosen for all elastic axial tests. Elastic axial testing of samples showed corroboration between sawbone and cadaveric samples (*Figure 1b*). Elastic axial testing and torsional testing confirmed FE model predictions. IFM exhibited a mean value of 1.526 mm and 0.901 mm for low and high stiffness respectively, for elastic axial testing. Cyclic fatigue testing showed plateaued deformation across 100,000 cycles for all groups (dynamized cadaver models showed in *Figures 1c/d*).

DISCUSSION: Insertion of the shims adjacent to the angled pins increased the stiffness by approximately 2-fold. Based on prior results from rat models, this is appropriate for enhanced bone healing by dynamization. Moreover, negligible inter-fragmentary movement differences of the fracture gap occurred during repeated load-cycling to mimic the projected lifecycle of the fixator while attached to the sheep. This shows stability of fixator across its life span and efficacy in a weight bearing animal. FE model results were generally in agreement with bench testing in key mechanical properties (construct stiffness and IFM). The model is being further developed to refine local stress analysis.

SIGNIFICANCE: The successful design, manufacture and characterization of this adjustable external fixator provides the means to evaluate the efficacy of dynamization and reverse dynamization in ovine models of bone healing. This fixator may be useful in small animal veterinary practice and could form the basis for a device suitable for use in humans.

ACKNOWLEDGEMENTS: We would like to acknowledge Lawrence Berglund for assistance with mechanical test designs and testing. This study was funded as part of a Department of Defense research grant (award number W81XWH-13-1-0324).

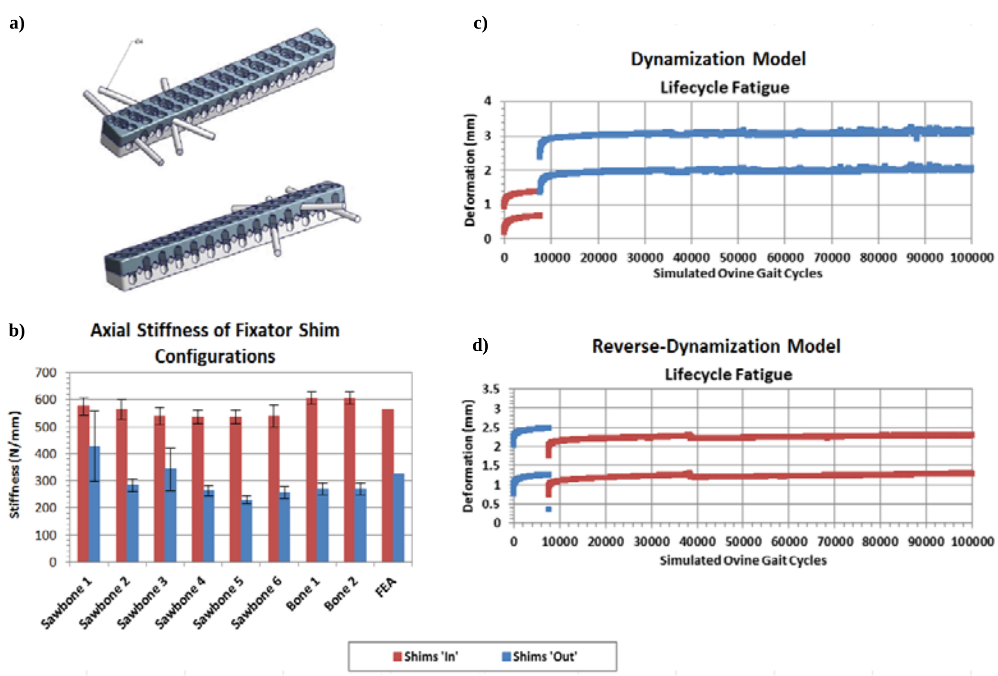


Figure 1 a): Novel external fixator design incorporating dual 'bodies' to compress surgical pins at variable positions. **b):** Measured axial stiffness of fixator configurations across sawbone / cadaveric ovine samples and FEA**. c) & d):** Min/Max deformation plots of three month lifecycle testing for both dynamizable models.

14. REFERENCES

[1] A. N. Pollak and J. R. Ficke, “Extremity war injuries: challenges in definitive reconstruction.,” *J. Am. Acad. Orthop. Surg.*, vol. 16, no. 11, pp. 628–634, 2008.

[2] E. J. Carragee, E. L. Hurwitz, and B. K. Weiner, “A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned.,” *Spine J.*, vol. 11, no. 6, pp. 471–491, 2011.

[3] V. Glatt, M. Miller, a Ivkovic, F. Liu, N. Parry, D. Griffin, M. Vrahas, and C. Evans, “Improved healing of large segmental defects in the rat femur by reverse dynamization in the presence of bone morphogenetic protein-2,” *J. bone Jt. Surg. Am. Vol.*, vol. 94, no. 22, pp. 2063–2073, 2012.

[4] M. Mehta, S. Checa, J. Lienau, D. Hutmacher, and G. N. Duda, “In vivo tracking of segmental bone defect healing reveals that callus patterning is related to early mechanical stimuli.,” *Eur. Cell. Mater.*, vol. 24, no. 0, pp. 358–71; discussion 371, 2012.

[5] G. De Bastiani, R. Aldegheri, and L. Renzi Brivio, “The treatment of fractures with a dynamic axial fixator.,” *J. Bone Joint Surg. Br.*, vol. 66, pp. 538–545, 1984.

[6] V. Glatt, C. H. Evans, and R. Matthys, “Design, characterisation and in vivo testing of a new, adjustable stiffness, external fixator for the rat femur.,” *Eur. Cell. Mater.*, vol. 23, pp. 289–98; discussion 299, 2012.

[7] V. Glatt, N. Loechel, N. Quirk, M. Schuetz, C. Evans, "Reverse dynamization: influence of fixator stiffness on the mode and efficiency of large bone defect healing at different doses of BMP-2." *J. bone Jt. Surg. Am. Vol.* 98: 677-687.

[8] R. J. Nesbitt, S. T. Herfat, D. V. Boguszewski, A. J. Engel, M. T. Galloway, and J. T. Shearn, “Primary and secondary restraints of human and ovine knees for simulated in vivo gait kinematics,” *J. Biomech.*, vol. 47, no. 9, pp. 2022–2027, 2014.

[9] C. A. Grant, M. Schuetz, and D. Epari, “Mechanical testing of internal fixation devices: A theoretical and practical examination of current methods,” *J. Biomech.*, Oct. 2015.

Improved Healing of Large, Osseous, Segmental Defects by Reverse Dynamization: Evaluation in a Sheep Model

Log number OR120192 / CCCRP R&A – December 2017
Award Number W81XWH-13-1-0324

PI: Evans, Christopher **Org:** Mayo Clinic **Award Amount:** $855,958

Study/Product Aim(s)

- To design, construct, characterize and evaluate a scalable, adjustable stiffness, external fixator that is appropriate for use in sheep and will allow reverse dynamization in a clinically expeditious manner.
- To evaluate the ability of reverse dynamization to enhance healing of a 3 cm, tibial defect in sheep.

Approach

We constructed an external fixator that can be applied to a fractured sheep tibia, allowing us to alter the stiffness of fixation while it is attached to the bone. The mechanical properties of the fixator were thoroughly evaluated and characterized. The final design was evaluated in a sheep, tibial segmental defect model None of 6 sheep in the study survived due to metal-ware failure. This occurred when startled sheep kicked, exerting high peak forces. Slinging and 24-hour CCTV monitoring failed to prevent this.

Animal x-rays indicating hardware failure. **Left)** Animal 1: Distal pin pullout. **Middle)** Animal 3: Proximal pin pullout**. Right)** Animal 5: Second proximal pin failure at thread-shaft interface.

Timeline and Cost

Activities CY	13	14	15	16	17 (NCE)
Fixator design & characterization					
Initiate in vivo ovine animal studies					
Complete in vivo data analysis					
Text (Major aim/study/milestone)					
Estimated Budget ($K)	$219,808	$334,274	$301,876		NCE

Goals/Milestones

The first specific aim was successfully accomplished with full mechanical characterization of the external fixator. By adding a strain gauge it was possible to measure in vivo forces exerted by sheep under these conditions.

The second aim of evaluating the effect of reverse dynamization on healing of a tibial segmental defect in sheep was not possible because of repeated metal-ware failures.

Budget Expenditure to Date: December, 2017
Projected Expenditure: $855,958
Actual Expenditure: $855,958

Updated: Mayo Clinic 12/2017